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FOOD ADDITIVES

Confirmatory Method for *N*-Nitrosodimethylamine and *N*-Nitrosopyrrolidine in Food by Multiple Ion Analysis with Gas Chromatography-Low Resolution Mass Spectrometry Before and After Ultraviolet Photolysis

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A confirmatory procedure is described for determining *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR) in bacon, beer, and malt by gas chromatography-low resolution quadrupole mass spectrometry (GC-MS). The presence of 3 monitored ions, m/z 30, 42, and 74 for NDMA, and m/z 30, 42, and 100 for NPYR, before and disappearance after photolysis under UV light at 365 nm without quantitation of peak areas was considered confirmatory evidence for their presence. The extracts, obtained by mineral oil distillation methods for bacon and malt, and dry column methods for bacon and beer, underwent cleanup procedures before capillary GC-MS analysis. Less than 100 ng nitrosamine in the total extract, or approximately 2 ng NDMA or 3 ng NPYR injected into the GC-MS instrument, can be confirmed by this method. This technique should also be applicable for other volatile nitrosamines.

When detected, volatile nitrosamines are generally present in trace amounts with potentially interfering substances which may lead to false positive results. Most nitrosamines have been shown to be carcinogenic toward many laboratory animal species (1). It is generally accepted that the presence of nitrosamines should be confirmed by mass spectrometry (MS). Many methods have been reported and these have been reviewed in detail (2, 3). While confirmation by gas chromatography (GC)-low resolution MS to yield a complete spectrum is the method of choice, this is not always possible. Most researchers using GC-high resolution MS consider observation of the molecular ion at the same retention time as the standard nitrosamine as the criterion for confirmation (2, 3). The disadvantage of high resolution MS is the cost, which may limit its availability for the routine confirmation of nitrosamines in foods analyzed by government, testing, industry, and university laboratories.

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GC-low resolution MS systems are more widely available than the high resolution instrument, but they require samples that are much cleaner. Guidelines for a hierarchy of MS confirmatory methods for animal drug residues have been reported (4) and these should be equally applicable to nitrosamines. The first choice is a full-scan spectrum, the second choice is a limited mass scan of a portion of the spectrum that contains only the characteristic information, and the final choice is the monitoring of 3 or more ions. For nitrosamines, the characteristic information is obtained from m/z 30, the ion for NO^+, to the parent ion, so that this will represent a limited scan mass spectrum. For any confirmatory method, the unknown and nitrosamine standard must have the same GC retention time.

Several reports have appeared in which a full-scan mass spectrum was obtained and reported for *N*-nitrosodimethylamine (NDMA) as a confirmatory method in processed fish (5), fish meal (6), malt (7), beer (7, 8), for NDMA and *N*-nitrosopyrrolidine (NPYR) in fried bacon and a beef-like product (9), and for NPYR in fried bacon (8). For multiple ion monitoring, the closer the ratios of the areas of the ions to be monitored are to that of the standard, the more reliable the confirmatory method will be. This method requires less sample than the full-scan mass spectrum method, but the sample must be free of co-eluting contaminants containing one or more of the ions monitored, or any contaminant containing one or more of the monitored ions that will interfere with integration of the peak areas. We believe that this difficulty can be overcome by taking advantage of the fact that nitrosamines are photolabile (10, 11), i.e., by using a procedure similar in principle to a photolytic method we reported as an additional aid to the confirmation of nitrosamines in which GC-thermal energy analyzer (GC-TEA) detection was employed (12). The samples are ana-

lyzed quantitatively for nitrosamines by GC-TEA, and then 3 principal characteristic ions of a nitrosamine are monitored by GC-MS before and after photolysis by UV light at 365 nm. We used this technique to confirm the presence of 3 volatile nitrosamines in a few samples in an earlier report (13). The general applicability and details of this technique for the determination of NPYR in fried bacon, and NDMA in malt and beer are reported here.

Experimental

Reagents

(a) *Solvents.*—Dichloromethane (DCM), hexane (dried over anhydrous Na_2SO_4), and pentane, all glass-distilled (Burdick & Jackson Laboratories, Muskegon, MI 49442).

(b) *Sodium sulfate.*—Anhydrous, granular, reagent grade (Mallinckrodt, Inc., St. Louis, MO 63147).

(c) *Silicic acid.*—100 mesh. Activate 4–5 h at 110°C, cool to room temperature, and deactivate with 5 g water/95 g silicic acid (Mallinckrodt, Inc.).

(d) *Alumina.*—Neutral, 100–200 mesh. Activate 3 h at 190°C, cool to room temperature, and deactivate to activity 3 with 6 g water/94 g alumina (Bio-Rad Laboratories, Rockville Center, NY 11571).

(e) *Boiling chips.*—Carborundum, small size (or equivalent).

(f) *N-Nitrosodimethylamine (NDMA).*—Working standard, 0.5 ng/μL DCM.

(g) *N-Nitrosopyrrolidine (NPYR).*—Working standard, 5.0 ng/μL DCM. Caution: NDMA and NPYR are potent animal carcinogens and must be handled appropriately.

Apparatus

(a) *Chromatographic columns.*—Condenser, 200 mm, with 19/38 joints top and bottom (A. H. Thomas Co., Philadelphia, PA 19105, Cat. No. 3215-M20). By glassblowing, lower joint was removed and replaced with stopcock. Also glass column, 15 mm id × 300 mm long with stopcock.

(b) *Evaporative concentrator.*—Kuderna-Danish, 250 mL with 4 mL concentrator tube and Snyder and micro-Snyder distilling columns (Kontes Glass, Vineland, NJ 08360, Cat. No. K-503000-0121, K-570001-0250, K-570050-425, K-569251-0319, or K-569001-0119).

(c) *L-Shaped tube and freezing trap.*—12/5 ball and socket joints with connecting tubing (A. H. Thomas Co., Cat. Nos. 5715-E46 and 5715-J46) were bent in an L-shape. By glassblowing, 19/38 male joint was connected to open end of ball joint, and open end of socket joint was connected to inlet tube of one-piece freezing trap, 195 mm long × 23 mm od.

(d) *Glass wool.*—Pyrex or equivalent.

(e) *Capillary tubes.*—Kimax 1.6–1.8 mm od × 45–50 mm.

(f) *Gas chromatograph–thermal energy analyzer (GC-TEA).*—Previously described (14).

(g) *Gas chromatography–mass spectrometry.*—Hewlett-Packard Model 5992B low resolution quadrupole GC-MS interfaced to HP-9825A desktop computer, HP-9866B printer, and HP-9885M flexible disk drive, and fitted with capillary interface system. GC instrument conditions: 30 m × 0.5 mm id glass capillary column coated with UCON 5100, or 25 m × 0.5 mm id glass capillary column coated with Carbowax 20 M; helium carrier gas 3.5 mL/min; injector 150°C; column 20°C for 2 min, programmed at 8°/min to 160°C, and held at this temperature. For NDMA, 3 ions, m/z 74, 42, and 30, and for NPYR, m/z 100, 42, and 30, were monitored under the following conditions: electron energy 70 eV; electron multiplier 2000–2600 eV; integration time 50 ms/mass monitored. When MS isolation valve was opened, 0.75 mL/min of He flow entered MS instrument and remainder was vented into overhead hood. Data acquired by GC-MS could be processed 1 ion individually or 3 ions simultaneously. More detail could be obtained from the former than from the latter; therefore, the data were processed 1 ion individually. Abundance scale for each ion differed from that of other ions.

(h) *UV source.*—Chromato-Vue Model C-5 (Ultra-Violet Products, Inc., San Gabriel, CA 91778). Medium intensity lamp with 284 microwatts/sq. cm of 365 nm radiation at distance of 457 mm.

Samples and Procedure

(a) Fried bacon samples containing various levels of NPYR were obtained from USDA, Food Safety and Inspection Service monitoring program of commercial producers. Nitrosamines were isolated by a reported modification (14) of a mineral oil distillation procedure (15) or a dry column procedure (16) and cleaned up by the following procedure: Add 15 mL hexane to water-cooled column and place glass wool plug in bottom. Add 5 g alumina and tap column to pack. (Cooling prevents vaporization of pentane and DCM on alumina column.) Top column with 1–2 cm Na_2SO_4. Drain most of hexane

and add 20 mL hexane to column. Add bacon extract, using 5 mL hexane to wash concentrator tube, and add wash to column. Technique of transfer should avoid contact with ground glass joint. Adjust stopcock to maintain flow rate of 1–2 mL/min, and then elute with 50 mL each of 1 + 10, 3 + 10, and 6 + 10 DCM–pentane and collect final fraction in Kuderna-Danish flask. Add boiling chip, fit flask with Synder column, and concentrate solvent on steam bath to 2–4 mL. Replace Synder column with micro-Synder column; then add boiling chip and concentrate solvent to 1.0 mL in 60°C water bath. (Recovery of NPYR varied from 77 to 100%.) With spatula, raise boiling chips above liquid level in tube, wash spatula and boiling chips with a few drops of DCM, and discard boiling chips. Cool extract to 10°C or lower (to prevent bumping) and connect tube to one end of L-shaped tubing containing 19/38 joint; connect other end of tubing, containing ball joint, to freezing trap which is cooled in dry ice–methanol bath and connected to laboratory vacuum system (50–90 mm Hg). Concentrate to 20–40 μL at ambient temperature. Transfer concentrate to 2 capillary tubes sealed at 1 end, and seal tubes. Place 1 tube 2–4 cm from UV light source at 365 nm for 3 h.

(**b**) Beer malt containing various levels of NDMA was obtained from the Food and Drug Administration. NDMA was isolated by a mineral oil distillation procedure (7), and cleaned up by the following procedure: Add 10 mL hexane to column and place glass wool plug in bottom. Add 1 g silicic acid as hexane slurry, wash sides of column with hexane, and open stopcock to pack column. Top column with 1–2 cm Na_2SO_4. Drain most of hexane, and add 20 mL additional hexane to column. Add malt extract to column, using 5 mL hexane to wash concentrator tube and adding wash to column. Open stopcock and wash column with 50 mL DCM–pentane (4 + 10), elute NDMA with 50 mL DCM, and collect eluate in a Kuderna-Danish flask. Concentrate sample as in (**a**). (Recovery of NDMA was 80–89%.)

(**c**) One brand of bock beer was obtained from a retail source and the NDMA was isolated by a dry column procedure (17). The extract was passed through an acid–Celite column (18) followed by procedure (**b**). (Recovery of NDMA was 89%.)

(**d**) A 2 μL sample injected into the GC–MS instrument which was maintained in the direct inject mode 0.6 min, in the flush mode 0.6 min, and finally in the run mode. NPYR was determined on the UCON 5100 column, and NDMA was determined from malt and beer on the Carbowax 20M column. GC retention times were 22.8–23.0 min for NPYR on the UCON 5100 column, and 14.6 min for NDMA on the Carbowax 20M column.

Results and Discussion

Initially, the nitrosamine peak was collected as it eluted from the GC column and then analyzed by GC-MS (13), but this technique was too time consuming as a cleanup procedure and was abandoned for a column chromatographic method. Silica gel, as used in the FDA multidetection procedure for volatile nitrosamines (19), was also used here, but ethyl ether, one of the solvents specified to elute nitrosamines, could not be used here because solvent contaminants interfered with the GC-MS analysis. Silicic acid partially deactivated with 5% water was used for cleanup of all extracts containing NDMA because DCM eluted this nitrosamine, but not NPYR. Elution profiles of volatile nitrosamines on activity 1–3 acidic, basic, and neutral Woelm alumina have been investigated and summarized (20). Based on that work, neutral alumina of activity 3 was selected for our investigation. With this column, NDMA eluted with DCM–pentane (3 + 10) mixture, whereas NPYR eluted with a 6 + 10 mixture. Although NDMA and NPYR could be isolated separately with this column, no attempt was made to confirm NDMA in fried bacon because this nitrosamine is usually present in concentrations much lower than the 10 ppb violative level established by the regulatory agencies.

The criteria for this GC–MS confirmatory analysis for NDMA and NPYR are the simultaneous presence before, and absence after, UV photolysis of the principal characteristic ions monitored and the same GC retention time.

In low resolution mass spectra of NDMA and NPYR in which the ions from m/z 29 to 120 were scanned, ions with abundances greater than 10% of the base peak were m/z 30, 42, 43, and 74 (base peak) for NDMA, and m/z 30, 39, 41 (base peak), 42, 43, 68, and 100 for NPYR. Three characteristic and intense ions were selected for multiple ion analysis. These ions also gave the 3 most intense peaks in the ion chromatograms, namely, the parent ion, m/z 74 or 100, and m/z 30 (NO^+) and 42 ($C_2H_4N^+$). The latter 2 are common ion fragments of volatile nitrosamines (21). Although m/z 41 was more intense than m/z 42 for NPYR in the limited scan mass spectrum, this advantage was nullified by its greater prevalence from the fragmentation of some of the extract

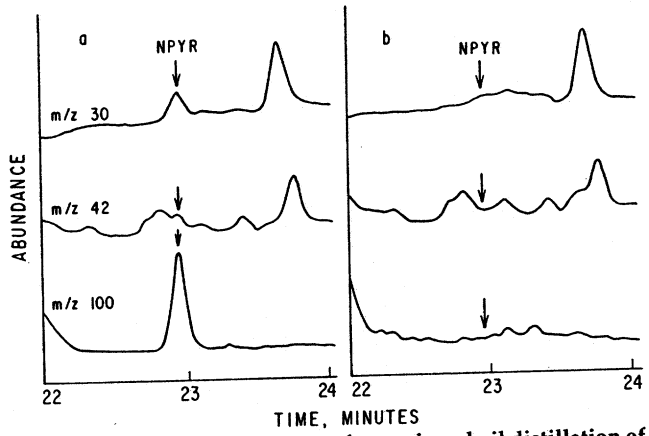

Figure 1. GC-MS of 3.7 ppb (92 ng) NPYR in extract from mineral oil distillation of fried bacon before (a) and after (b) UV photolysis.

contaminants when low levels of NPYR were analyzed.

The selected ion current profiles from the 3-ion monitoring of extracts from bacon and beer for NPYR or NDMA before (a) and after (b) photolysis under UV light are shown in Figures 1–3. The abundance scale for any given ion before and after photolysis differed by 20% or less except for m/z 100 in Figure 1, and m/z 74 for Figure 2, both of which differed by 70%, and for m/z 74 in Figure 3 which differed by 60%. The latter 3 are the result of the disappearance of the large nitrosamine peak after photolysis, with a corresponding decrease in the ion abundance scale. There were large variations in the ion abundance scale for different ions, therefore direct comparison of peak areas cannot be made.

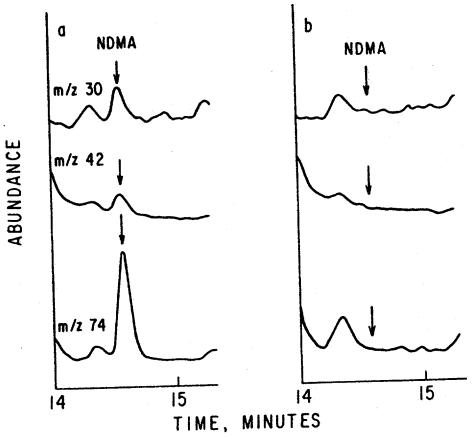

Figure 2. GC-MS of 1.5 ppb (73 ng) NDMA in extract from dry column treatment of beer before (a) and after (b) UV photolysis.

Bacon sample analyzed by the mineral oil distillation procedure and containing 3.7 ppb NPYR or a total of 92 ng NPYR is shown in Figure 1. The chromatogram represents an aliquot containing approximately 3 ng NPYR that was injected into the GC–MS system. The m/z 42 ion peak for NPYR is on the shoulder of a larger peak but it is evident that this as well as the other 2 peaks disappeared after photolysis. Two other peaks for m/z 42 at different retention times increased in size after photolysis, but did not interfere with the interpretation of the results. In fried bacon samples containing greater than 5 ppb NPYR, the intensity of all the ion peaks increased, particularly the m/z 42 peak. As the level of NPYR decreased to 5 ppb and below, the ions of m/z 42 and 30 for NPYR began to disappear into the background and became less discernible, while the m/z 100 ion peak remained strong because of the low background level for this ion. A more rigorous cleanup procedure than the one reported here should significantly decrease the background level, especially for m/z 42 and 30, thereby increasing the sensitivity of the procedure. When fried bacon was analyzed by the mineral oil distillation procedure, NPYR levels of 80–90 ng/25 g appeared to be the lower detection limit by this method. For fried bacon analyzed by the dry column method, extracts containing lower total NPYR than that from the mineral oil distillation procedure, 44 ng/10 g or 4.4 ppb, could be confirmed. The reasons were less ion fragments due to contaminants and therefore lower background levels for ions of

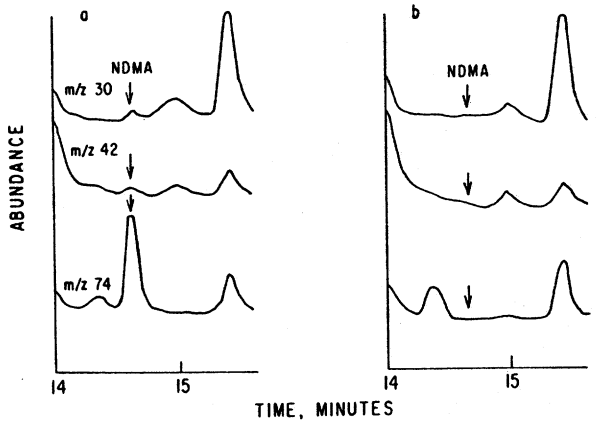

Figure 3. GC-MS of 2.8 ppb (71 ng) NDMA in extract from mineral oil distillation of malt before (a) and after (b) UV photolysis.

m/z 30 and 42, and probably less NPYR was lost during the concentration step. The tube containing the extract was cooled by the vaporization of the solvent during the concentration step and a thin film of solid, possibly mono- and diglycerides, which precipitated on the walls of the tube, melted at room temperature and was dissolved by washing the walls of the tube with the concentrate. This oily film probably decreased the loss of NPYR during the concentration step. On injection into the GC-MS system, these oils condensed on the walls of the injection port liner.

Figures 2 and 3 represent a bock beer extract containing 1.5 ppb or a total of 73 ng NDMA, and a malt sample analyzed by the mineral oil distillation procedure and containing 2.8 ppb NDMA or a total of 71 ng NDMA, respectively. In the chromatogram, which represents an injected sample containing approximately 2 ng NDMA, the presence before (a) and absence after (b) UV photolysis of NDMA is evident. The lower limit of detection for malt with the isolation procedure used was probably about 70 ng NDMA/25 g. For beer, the detection limit is not known but should be below 70 ng NDMA in the extract. The Food and Drug Administration recently established a NDMA violative level of 5 ppb for malt beverages (22) and >10 ppb for malt (23).

Extracts from malt contained a large peak that eluted just before NDMA on the UCON 5100 column, which interfered with the analysis. However, with the Carbowax 20M column, the elution time of this peak was about 1 min before the NDMA peak and could be eliminated from the ion chromatogram. The beer extract also contained interfering peaks that were present when the sample cleanup was performed by the acid-Celite or silicic acid columns, but the use of both columns eliminated this interference.

Ion peaks from non-nitroso and nonphotolyzable compounds were always present in the chromatograms. By using these peaks as internal reference standards, slight variations in the amount of the before and after photolysis samples injected into the GC-MS system, or abundance scale for a given ion chromatogram, did not interfere with the interpretation of the results.

This GC–MS procedure was applied successfully to the samples shown in Table 1. The greatest emphasis was placed on bacon because this was the foodstuff in which nitrosamines have been detected consistently. At present, fried bacon is routinely analyzed by the mineral oil distillation procedure for volatile nitrosamines; therefore, the greatest emphasis was placed on applying this GC-MS technique to extracts obtained by that isolation procedure. A few samples were analyzed by a recently developed dry column method (16) because this method appears to be more reliable and faster than the mineral oil distillation procedure and may find wider use for the routine analysis of NPYR in fried bacon.

The major advantage of this low resolution GC-MS confirmatory procedure is the low levels of nitrosamines required; the major disadvantage is that 2 analyses are required for the confirmation of one sample. This procedure will be suitable when the amount of sample is inadequate for a limited or full scan mass spectrum or a multiple ion analysis in which the ratios of the